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Biomimetic Synthesis and Structural Revision of Chaxine B and Its Analogues

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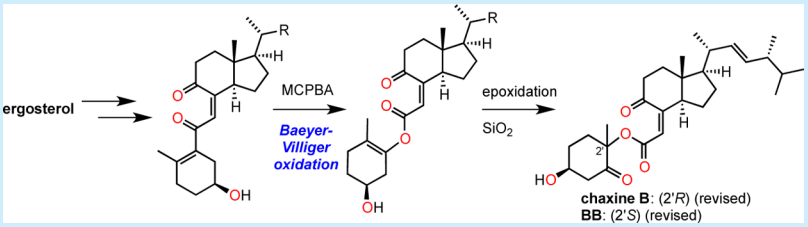
Supporting Information



ABSTRACT: Chaxine B and its analogues were synthesized from ergosterol in eight steps on the basis of our proposed biosynthetic pathway, which includes a highly site-selective and regioselective Baeyer–Villiger oxidation as the key step. This synthesis enabled the revision of the structures of chaxine B and its analogues.

Chaxine B and its analogues, chaxine C, D, E, and A, as well as demethylincisterol A_3, were isolated from *Agrocybe chaxingu*, an edible mushroom that grows only at high altitude in southern China (Figure 1).[1] Chaxine B was also isolated

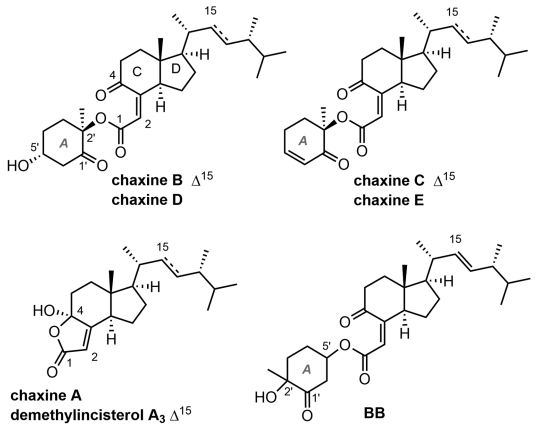


Figure 1. Originally proposed structures of chaxines A–E, demethylincisterol A_3, and BB (the assignment of the absolute stereochemistry of the A rings is tentative).[1]

from *Penicillium* sp. together with related compound BB.[2] Interestingly, demethylincisterol A_3, a carboxylic acid moiety of chaxine B and C, was also isolated from other natural sources, such as mushrooms, fungi,[3] and marine animals.[4] Chaxines show potent suppressive activity toward the formation of osteroclast, while BB and chaxine B accelerate neural stem cell propagation. Despite such medicinally important properties, details of the biological investigations have so far been hampered by the limited quantities of these compounds that are available from natural sources.[5] The structures of these compounds were elucidated by an extensive analysis of 2D NMR and MS spectra, which revealed unprecedented and highly degraded steroid structures, in which the A and C/D rings are connected by an ester linkage. Although the relative stereochemistry between C2′ and C5′ in the A ring of chaxine B and D was elucidated, its absolute configuration remains to be determined. In the case of BB, even the relative stereochemistry of the A ring has not yet been clarified. The syntheses of chaxine A and demethylincisterol A_3 from ergocalciferol (vitamin D_2) have already been reported,[6] while no synthetic studies of an ester type such as BB or chaxines B–E have been reported, probably due to their stereochemical ambiguity. Herein, we propose that these chaxines might be biosynthesized from ergosterol (**1** in Scheme 1), which is an abundant steroid in mushrooms, based on the structural similarity of their side chains. This assumption enabled us to propose the absolute stereochemistry of the A ring as shown in Scheme 1. In order to confirm this stereostructure, we planned to synthesize chaxine B (denoted **8**) from ergosterol (**1**) on the basis of a route inspired by our proposed biosynthetic route outlined below.

Scheme 1 shows our proposed biosynthetic route to chaxine B from ergosterol. Photoinduced electrocyclization of ergosterol (**1**), a well-known reaction in the biosynthesis of vitamin D_2, provides a mixture of isomers, in which previtamin D_2 (**2**) or tachysterol (**3**) may be considered as a biosynthetic intermediate of chaxines. One of these two compounds may be oxygenated to enedione **5** through e.g. endoperoxide **4**, which can be obtained by a [4 + 2] cycloaddition with singlet

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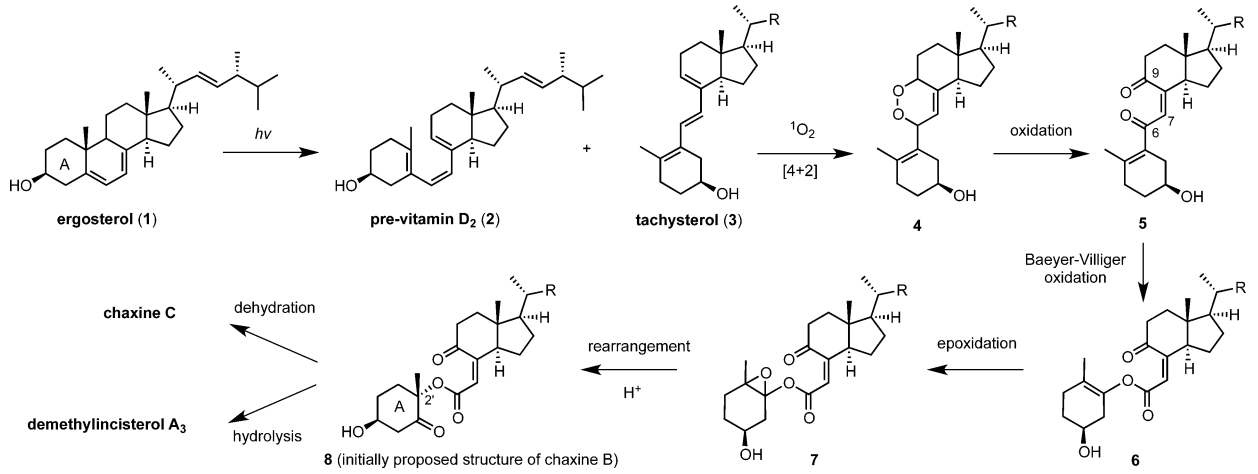
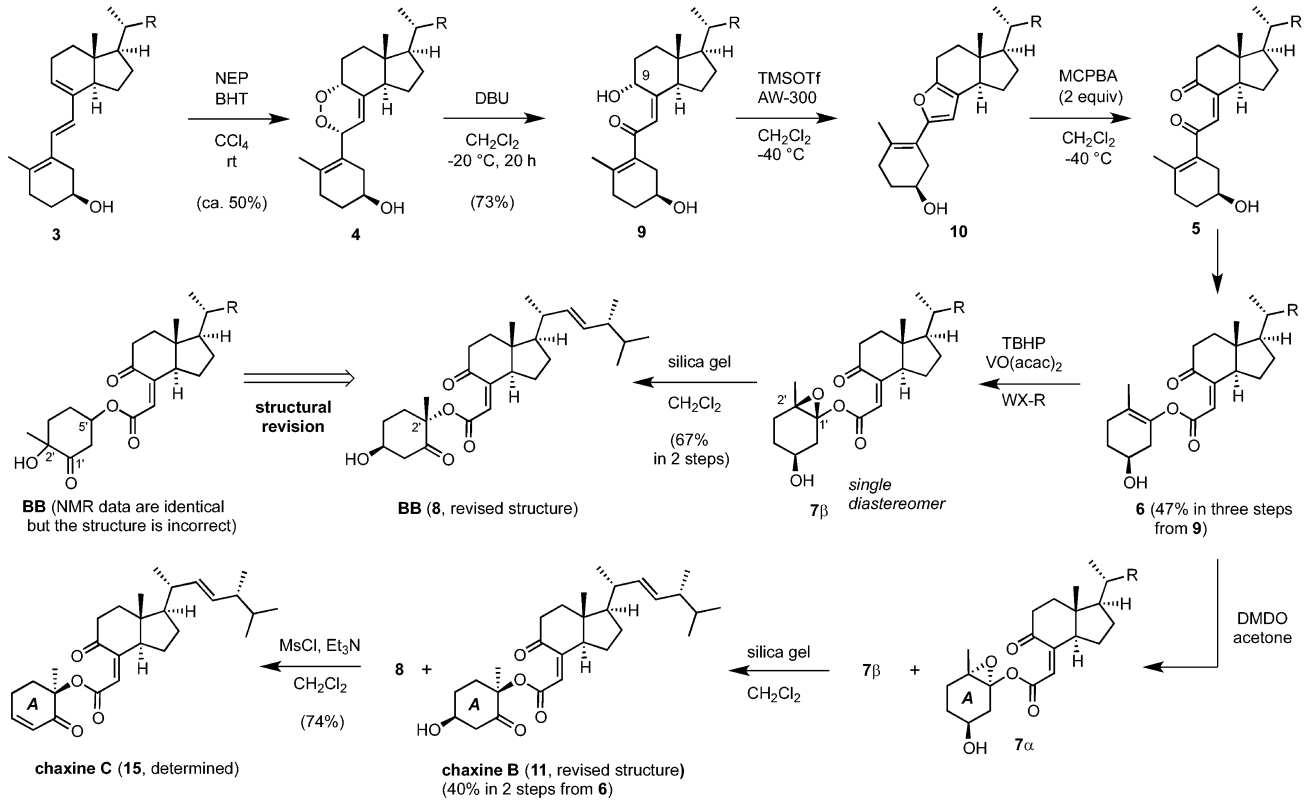
Scheme 1. Proposed Biosynthetic Route to Chaxine B and Related Compounds from Ergosterol (1)



Scheme 2. Synthesis of BB, Chaxine B and C, and Their Structural Revision



oxygen ($^1O_2$). A subsequent Baeyer–Villiger oxidation of **5** at the C-6 ketone may generate enol ester **6**. Epoxidation of the electron-rich enol ester moiety, followed by acyloxy migration under acidic conditions, should then afford chaxine B (**8**) or its diastereomer at the C-2′ position, which should depend on the stereochemistry of the epoxide of **7**. Chaxine C and demethylincisterol A_3 should then be obtained from **8** upon dehydration or hydrolysis, respectively.[7]

In order to emulate the biosynthetic route chemically in the laboratory, three synthetic challenges have to be resolved, although those may be managed by enzymes of the producers; (i) the site-selective and regioselective Baeyer–Villiger oxidation of **5**, (ii) the diastereoselective epoxidation of **6**, and (iii) the stereochemistry of the rearrangement of the enol ester epoxide **7**. To the best of our knowledge, the Baeyer–Villiger oxidation of a complex system such as **5** has not yet been reported.[8,9] The site-selectivity and regioselectivity of the Baeyer–Villiger oxidation of **5** are difficult to predict, as they depend on many factors, e.g. the electron density and steric bulk of the substituents and stereoelectronic effects. In this specific case, however, the two ketones connected via the alkene group might behave independently, considering that the planar structure required for conjugation should be difficult to attain on account of the steric and electronic repulsion between the two ketone moieties. As the migration step is the rate-determining step of the Baeyer–Villiger oxidation, we assumed that the migratory aptitude of the substituents might determine the site-selectivity and regioselectivity of the reaction. As the

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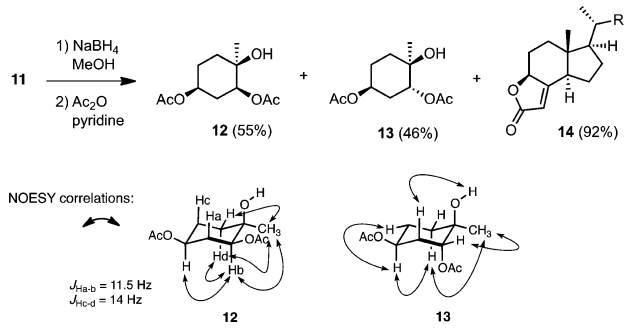
tetrasubstituted alkene of the A ring possesses the highest migratory ability among the four possible substituents, we anticipated the Baeyer–Villiger oxidation of **5** to occur preferentially at the C-6 ketone to provide the desired product **6**. The stereochemistry of the epoxidation of **6** could be controlled by a metal-assisted catalysis of homoallylic alcohol or by the steric hindrance arising from the protection of the hydroxy group.[10] Since the silica-gel-promoted rearrangement of enol ester epoxides has been reported to be highly stereoselective,[11] one of the two diastereomeric epoxides **7** should afford chaxine B, a natural product, which should enable the unambiguous determination of the stereostructure of the A ring. With these considerations in mind, we embarked on the synthesis of chaxine B and its analogues.

Tachysterol (**3**), rather than previtamin D_2 (**2**), was chosen as the starting material due to its thermal stability and suitability as a substrate for the oxygenation with $^1O_2$ (Scheme 2). Photochemical isomerization of ergosterol (**1**) affords a mixture of previtamin D_2 (**2**), tachysterol (**3**), and lumisterol, whereby the composition of the mixture depends on the wavelength of the UV light:[12] irradiation with short-wavelength UV light (λ = 254 nm) by a low-pressure mercury lamp furnished **3** in 55% yield as the major product. However, the subsequent reaction of **3** with $^1O_2$ proved to be problematic: endoperoxide **4** was obtained in poor yield (<35%) under standard conditions using light and tetraphenylporphyrin (TPP) as a sensitizer, probably due to overoxidation of the product. In order to suppress the side reactions, we attempted the reaction with dimethyl naphthalene-endoperoxide (NEP),[13] which has previously been reported to generate $^1O_2$ quantitatively at room temperature. Treatment of **3** with NEP (5.5 equiv) in the presence of di-*tert*-butylhydroxytoluene (BHT) at room temperature improved the yield of **4** to ca. 50%.[14] In order to transform **4** into enedione **5**, we initially attempted a two-step procedure comprising the cleavage of the endoperoxide moiety followed by oxidation. Endoperoxide **4** was treated with DBU to give **9** as a single product;[15] however, the selective oxidation of the allylic hydroxy group at the C-9 position of **9** was unsuccessful. Therefore, we synthesized enedione **5** by oxidation of furan **10**, which was easily prepared from **9** using the Paal–Knorr method. Treatment of **9** with TMSOTf in the presence of molecular sieves (AW-300) afforded **10** in high yield as an unstable product. Without purification, **10** was oxidized with MCPBA at −40 °C, and to our delight, we observed the formation of enedione **5** as well as enol ester **6**, which is the desired product of the Baeyer–Villiger oxidation of **5**. The oxidation of **10** with 2.1 equiv of MCPBA furnished enol ester **6** in 47% yield in three steps from **9**. Therefore, the site-selective and regioselective Baeyer–Villiger oxidation of **5** was successfully accomplished as anticipated.

Subsequently, we investigated the regio- and stereoselectivity of the epoxidation of **6**. Reaction of **6** with TBHP and a catalytic amount of $VO(acac)_2$[10,16] provided epoxide **7*β*** as a single diastereomer,[17] which was subsequently treated with silica gel to give a crystalline product in 67% overall yield from **6**. The ^1H and ^{13}C NMR spectra of this product were identical to those of BB. However, an X-ray crystallographic analysis revealed that its structure is not consistent with a structure that contains an ester of the secondary alcohol, as reported, but with the structure of **8**, which contains an ester of the tertiary alcohol of the A ring.[18] Confusingly, the relative stereochemistry of the A ring in **8** is identical to that originally assigned to chaxine B (see Figure 1). This result clearly suggests that the structure of chaxine B needs to be revised.

As the silica-gel-promoted rearrangement of **7*β*** proceeded highly stereoselectively to afford **8**,[19] we expected that the corresponding *α*-epoxide **7*α*** would provide an epimer at the C-2′ position of **8**, i.e. the revised structure of chaxine B. We therefore explored the optimum epoxidation conditions giving **7*α*** as the major product. Epoxidation with MCPBA in Et_2O furnished **7*β*** as a single diastereomer.[20] After many experiments, we found that oxidation of **6** with dimethyldioxirane (DMDO) in acetone afforded a diastereomeric mixture of epoxides **7*β*** and **7*α*** (ratio = ca. 3:5 by ^1H NMR analysis), which was directly exposed to silica gel to afford **8** and **11** in 21% and 40% yield, respectively. The NMR spectra of **11** are identical to the reported data for chaxine B. Unfortunately, **11** (chaxine B) could not be crystallized, and the stereochemistry of the A ring of chaxine B had thus to be confirmed by degradation experiments (Scheme 3). Reduction of the

Scheme 3. Chemical Degradation of Chaxine B (11) and the Stereostructure of the A Ring



synthesized chaxine B with $NaBH_4$, followed by acetylation, furnished a mixture of **12**, **13**, and **14**.[6a] Their structures, including the relative stereochemistry of **12** and **13**, which bear A rings, were determined by extensive analysis of their 2D-NMR spectra and are shown in the Scheme 3.[21] We thus conclude that the correct structure of chaxine B is **11**, an epimer at the C-2′ position of BB (**8**). Furthermore, we determined the structure of chaxine C, by dehydration (MsCl, Et_3N) of **8** and chaxine B (**11**), which afforded **16** (not shown) and **15** in 92% and 74% yield, respectively. Comparison of the ^1H and ^{13}C NMR spectra of **16** and **15** with those of natural chaxine C revealed that **15** is identical with chaxine C (Scheme 2), which strongly implies that chaxine C is, as proposed, biosynthetically derived from chaxine B. Therefore, we propose that the stereochemistry of the A ring of chaxine D and E is identical to that of chaxine B and C, respectively.

In summary, we have achieved the eight-step synthesis of chaxine B and its epimer BB from ergosterol, which was inspired by our biosynthetic proposal of these natural products. Unexpectedly, the X-ray analysis of BB resulted in a revision of its previously reported structure to that of **8**, while the structure of chaxine B was revised to that of **11**, which was based on chemical degradation experiments and analyses of the 2D NMR spectra (Figure 2). The salient feature of this synthesis is the oxidative cascade of furan **10** to enol ester **6** with MCPBA, which includes the formation of enedione and a highly site-selective and regioselective Baeyer–Villiger oxidation. Unfortunately, an attempted concomitant epoxidation of the enol ester under the same conditions was unsuccessful due to competitive

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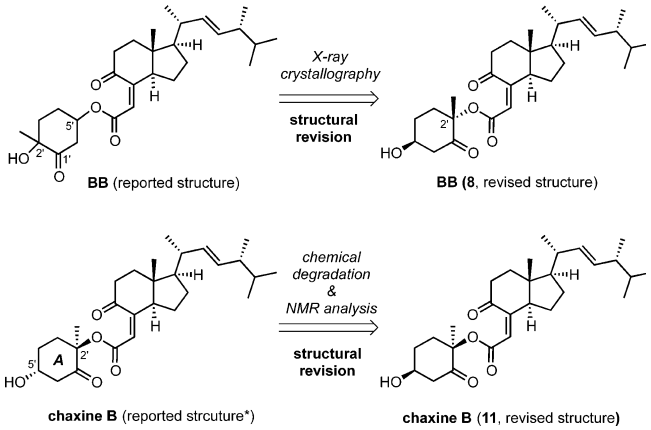

Figure 2. Summary of the structural revision of chaxine B and BB (*the assignment of the absolute stereochemistry of the A rings is tentative).

epoxidation of the side chain. Since this synthetic route is very short (operationally seven steps), it should provide a variety of chaxine analogues for biological investigations. This study thus strongly supports the notion that ergosterol is the biosynthetic precursor of chaxines and that the synthetic route presented herein might indeed be similar to the actual biosynthetic route. Therefore, these results should contribute to the elucidation of the biosynthesis of chaxines in the near future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03724.

Experimental procedures, characterization data, copies of ^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(4) Mansoor, T. A.; Hong, J.; Lee, C.-O.; Bae, S.-J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2005**, *68*, 331.

(5) Chaxine B, C, D, and E were isolated in 2.1, 1.6, 0.5, and 0.4 mg respectively from 1.5 kg of dried fruiting bodies of the mushroom.

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(14) The stereochemistry of the endoperoxide of **4** was determined by NOESY correlation between the proton at the C-9 and the angular methyl protons.

(15) We expected that the Baeyer–Villiger oxidation of **9** might occur selectively at the C-6 ketone at this stage; however, the attempted Baeyer–Villiger oxidation with MCPBA resulted in an epoxidation of the A ring in a good yield.

(16) Jeker, O. F.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2012**, *51*, 3474.

(17) The diastereoselectivity was determined by ^{1}H NMR analysis of the crude product.

(18) CCDC-1508011 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(19) Judging from the structures of **7β** and BB (**8**), the acyloxy migration proceeded with inversion of the configuration at the C-2′ position, which is consistent with the conclusions of ref 11.

(20) The epoxidation with MCPBA in CH_2Cl_2 as a conventional solvent gave a substantial amount of byproducts, including epoxides of the side chain.

(21) Details of the structure determination are included in the Supporting Information.

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